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Original Article

An Investigation of the Establishment, Culture and Essential Oil Composition of *Hyssopus Officinalis* in Dry and Irrigated Farming in Barren Land

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Abstract

Background and Aim: *Hyssopus officinalis L.* belonging to the family *Lamiaceaeis* a perennial herb known as a culinary and medicinal herb. This study was conducted to investigate the compatibility of culture and the effect of drought on the growth and essential oil compounds of hyssop in Lorestan province, Iran.
Materials and Methods: In this study, we first evaluated the ability of hyssop plant with regard to adaption and establishment in Aligudarz region in Lorestan province. Then, hyssop was cultivated in irrigated and non-irrigated (dryland) conditions, and their essential oil changes were investigated.
Results: The results of the present study showed that the fresh and dry weight of established plants was 10590 and 3780 kg. ha^{-1} respectively ,which indicates the compatibility of hyssop plants in Aligudarz region. Moreover, the results showed that 95.8% of essential oil composition was determined in irrigated conditions that the highest amount of which was related to Isocamfopinone with 34.2%. Furthermore, the percentage of compounds such as Trans-pinocamphone (19%), Thymol (28.8%), Ortho-menth-8-ene-4-methanol (6.86%), ß–pinene (6.35%), Myrtenol (4.18%) and Pinocarvone (3.84%) had the highest amount of essential oil composition compared to other essential oil compounds. In dryland conditions, about 97.39% of the total essential oil compositions were identified. Also, it was found that the highest amount of these compounds was related to Isocamphopinone (38.63%).
Conclusion: It was found in this study that irrigated and dryland conditions had different effects on the essential oil compounds of the hyssop medicinal plant so that in both conditions some compounds increased and others decreased, and some were constant. Moreover, depending on the production goal, hyssop can be cultivated in each of these conditions.
Keywords: Essential oil, Isocamfopinone, Irrigated farming, Medicinal plants

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Introduction

Hyssop (*Hyssopus officinalis* L.) is one of the most important medicinal plants belonging to family *Lamiaceae* that grows in western Asia, in the districts around the Caspian Sea and Southern Europe (1).

Hyssop is widely used in traditional medicine. Hyssop oil may exist as flavour component in a variety of food products. The oil is an aromatic ingredient in soaps, cosmetics and perfumes (2). Since hyssop plant essential oil contains a variety of chemical compounds, it is one of the most significant medicinal herbs used in food, health and cosmetic industries throughout the world. In traditional medicine, this plant is utilized in the treatment of bronchitis and respiratory infections, particularly when production of mucus is high. It is also used as a sedative to treat asthma in kids and adults (3). Hyssop that contains a remarkable amount of volatile oil, flavonoids, tannins, and marrubin, has been used as a healing plant to make digestive disorders, cure laryngitis less severe. Moreover, it has been used to expedite wound healing in Turkish traditional medicine (4).

Essential oil is one of the main groups of biologically active compounds (5). It has also been mentioned that oil mainly accumulates in the flowers and leaves, whereas its amounts in the stems are noticeable. Essential oils obtained from the aerial parts of *H. officinalis* are widely used in pharmaceutical and/or food industries in order to make medications for illnesses of the respiratory system or as a spice to flavor foods (1). Also, it has already been reported that the essential oil of this species has antiviral and antibacterial activities (6). Hyssop is fragrant, and an essential oil, characterized primarily by the existence of monoterpenes, can be extracted from its aerial parts. Most of references refer to pinocamphone (Cis and Trans) as the major compounds while β-pinene and pinocarvone occur frequently in high concentrations (7). Sesquiterpenes are less abundant, among which germacrene-D appears to be the most prevalent constituent (NémethZámbori, 2015). Isopinocamphone (43.29%) is the main component of hyssop (8). The main components of essential oil in hyssop were reported as Pinocamphone (49.1%) by Garg *et al.* (9) and Pinocarvone (36.3%) by Ozer H *et al*. (10).

Irrigated and dryland conditions had different effects on essential oils compounds of the medicinal plants. In Hyssop medicinal plant cultivated in Iran, in the irrigation condition of 60% field capacity, 27 compounds were extracted from the essential oil of Hyssop which are equal to 96.97% of total compounds and also in the irrigation conditions of 40% field capacity, 42 compounds were identified that are equal to 99.27% of total essential oil of the sample (11). It has also been reported that 18 out of 31 compounds of the composition of the essential oil obtained in normal irrigation conditions were found with different values in conditions of 60% and 40% field capacities. The main components of *H. officinalis* oil were cis-pinocamphone (26.85 %), β-pinene (20.43 %), trans-pinocamphone (15.97%), α- elemol (7.96 %), durenol (3.11%), β-phellandrene (2.41%), caryophyllene (2.34%), (E)-2,6-dimethyl-1,3,5,7-octatetraene (2.27%), 3(10)-caren-4-ol,acetoacetic acid ester (2.14%), bicyclogermacrene (1.83%), myrtenol (1.73%), germacrene-D (1.68%), limonene (1.56%), γ-eudesmol (1.36%) and linalool (1.08%) (Hussein *et al*. 2015). The results showed that in *H.officinalis* L., all the samples in both irrigated and unirrigated models, contained the two major terpenes, i.e. pinocamphone and Iso-pinocamphone (35-40%) (12). However, the most prominent compound of hyssop was reported to be Myrtenyl acetate (74.08%) in another study (Fathizad *et al*., 2011). Moreover, Thymol (18.95%), N-decane (11.76%), Bisabolol (10.62%) and Carvacrol (7.73%) were mentioned as the main compounds of hyssop (15). These distinctions in the essential oil compositions could be related to various environmental determinants such as climatic, seasonal and geographical or ontogenesis variations. Therefore, this study was conducted in order to evaluate the establishment and growth of hyssop in Aligudarz region and also to determine the essential oil components of hyssop in dryland and irrigated conditions.

Materials and Methods

The present study aimed to investigate the cultivation of different ecotypes of medicinal plant of hyssop (endemic ecotype) in degraded areas of Lorestan province. The compatibility experiment and yield of Hyssop medicinal plant were laid out in randomized complete blocks design with 3 replications. Treatments depended on water availability, i.e. dryland and irrigated conditions. Figure 1 shows hyssop and its farm at Aligudarz region. The experiments for evaluation of essential oil composition changes under dryland (with 350 mm rainfall during growth season) and complete irrigation (during growth season from cultivation until harvesting) were performed in 2018 and 2019. For this purpose, Latitude regions of the province and its height from sea level were measured using the GPS device. Subsequently, coordinates with UTM Convert software were converted to UTM (Figure 2). The experiment site was located in Azna Mahlamak rural region, Aligudarz, Lorestan province, Iran. The altitude was 2570m and X=393294.55 and Y= 3668123.15 with about 15 degree of slop. The region of experiment had a cold climate with high rainfall at winter season.

Extraction of the Essential Oil

Sampling was carried out in the early flowering stage and all the samples were taken in both juvenile flower and leaves. In order to extract hyssop essential oil, the hydro distillation method with a Clevenger-type apparatus was used on air-dried plant materials, and the procedure took 3 hours. Separated materials were kept in a firmly sealed vial in 4°C until they were analyzed (13).

Chromatography Analysis

Analytical gas chromatography of the essential oil for hyssop was performed using a Hewlett-Packard 5975B series gas chromatograph with Agilent HP-5 capillary column (30 m×0.25 mm, f.t 0.25 µm); the carrier gas was Heand split ratio of 1:10, and a flame ionization detector was used (Figure 3). Temperature of the column was adjusted at 50°C which remained fixed for 10 min and was planned in order to increase to 240°C at the rate of 4°C/min and stay unchanging at that temperature for 15 min. GC/MS analysis was carried out on a HP 5975B having a Hewlett–Packard 5973 quadruple detector, on a HP-5 (30 m×0.25 mm; f.t 0.25 µm) capillary column. The MS operated at 70 eV ionization energy. Retention indexes were all calculated using the retention time of n-alkenes which were injected following the volatile oil at identical chromatographic conditions. Quantitative data were acquired from the electronic integration of the FID peak areas. Oils components were categorized via making comparisons their mass spectra and kovats indexes with published books and the Wiley library, available data bases and credible websites (13).

Results and Discussion

Chemical analyses of the soil and hyssop cultivation site in Aligudarz have been shown in (table 1). The soil sampling was from 30cm depth and clay, silt and sand percentage in soil sample were 27, 38 and 38% respectively, and soil texture was loam sand.

Loam sand soil texture does not have ventilation problems. With regard to the appropriateness of the organic matter percentage in soil organic carbon, it is essential to be added for the normal growth of hyssop. Phosphorus available in the soil is less than the appropriate range. The amounts of manganese and copper in the soil are in the optimum range but the amount of Mn in the studied soil was less than normal.

Levels of iron and zinc in the soil were less than the proposed range for the normal growth of medicinal plants.

Fresh and Dry Yield Plant Materials of Hyssop

Figure 1. The hyssop and its farm in Aligudarz region.

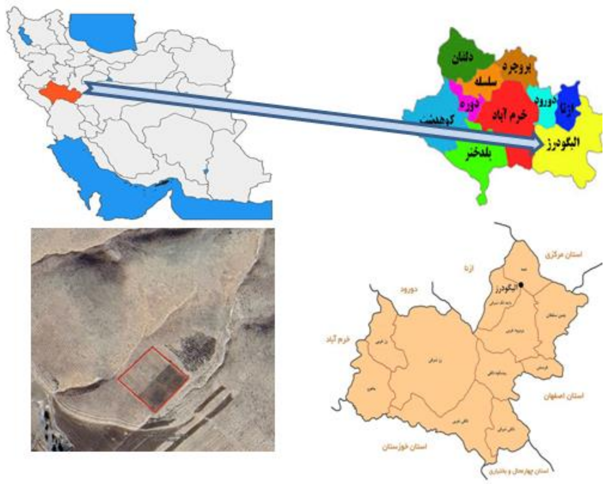

Figure 2. Geographical Map Location of the Experiment Area.

Figure 3. Gas Chromatography and Mass Spectrometry Devices.

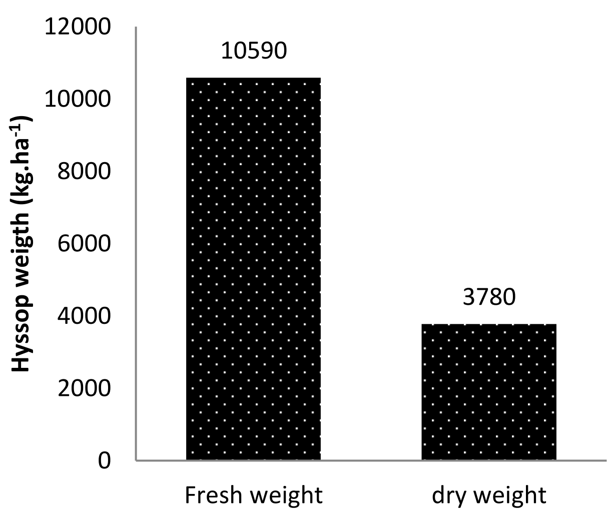

Figure 4. Average Yield of Fresh and Dry Weights of Hyssop for Establishment under Degraded Rangelands.

Providing data on the establishment and survival of hyssop in Aligudarz region was successfully accomplished, and growth of hyssop was normal. After establishment and growth, fresh and dry weights were

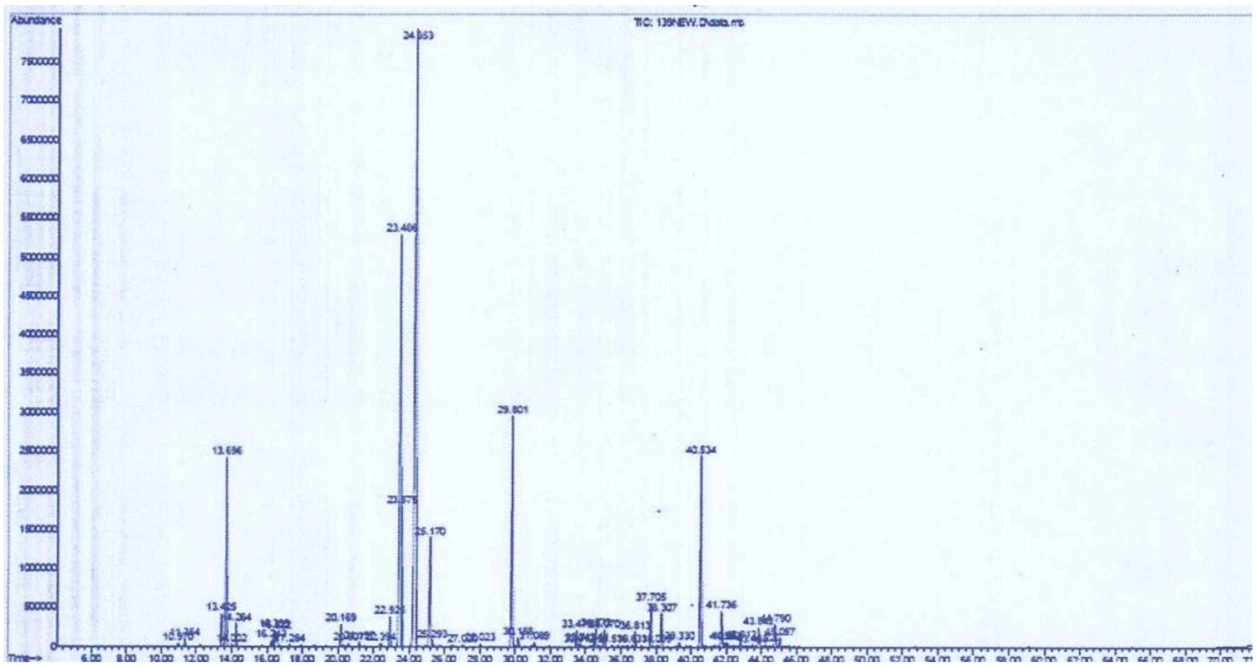

Figure 5. Hyssop Essential Oil Chromatography in Irrigated Conditions.

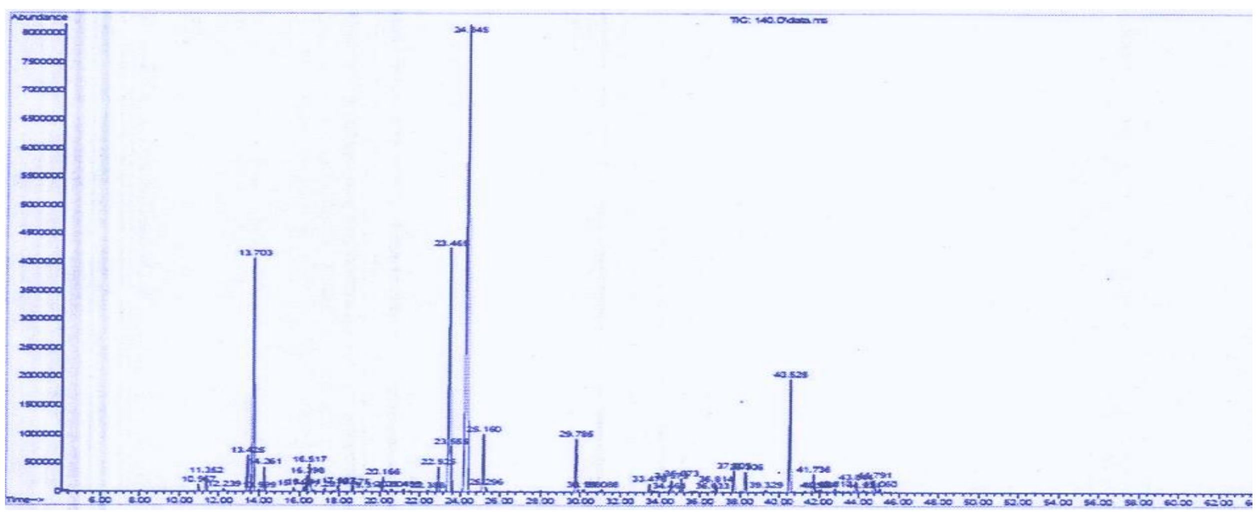

Figure 6. Hyssop essential oil Chromatography in dryland conditions.

Table 1: Results of Soil Analysis in the Cultivation Site of Hyssop Medicinal Plants.

Soil texture	pH	OC (%)	Total N (%)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Loam sand	6.97	1.4	0.59	21.6	810	3	0.38	0.22	8.6

measured. Based on the results, it was found that fresh and dry vegetative biomasses of hyssop were 9280 and 3780 kg. ha^{-1} respectively under degraded rangelands (figure 4).

There are some differences in dry weight between various medicinal herbs as well as between environmental conditions and the growth potential of different medicinal plants. One reason for the difference in growth characteristics and morphology of plants is variations in the plant growing environment. The environment is very important in creating diversity in plants (14).

After the establishment experiment, the essential oil composition was studied under two dryland and irrigated conditions after the cultivation of hyssop in both regions.

Essential oil Composition

According to the results of this study, about 95.8% of hyssop essential oil compounds (38 compositions) were identified in hyssop flowers in irrigated conditions. In this condition, the highest amount was related to isocamfopinone (34.2%). Also, compounds such as trans-pinocamphone (19%), thymol (28%), ortho-menth-8-ene-4-methanol (6.86%), ß-pinene (6.35%), myrtenol (4.17%) and pinocarvone (3.86%) had the highest amounts of essential oil composition compared to other essential oil compounds. Moreover, it was found that among all the identified compounds in hyssop essential oil in irrigated conditions, the lowest amounts of components were related to ß-ocimene, thymyl methyl ether, ά-humulene and δ-eudesmol, which had values less than 1% among the constituents of hyssop essential oil (Table 2).

In the case of hyssop, about 97.39% of the total compounds in the hyssop flower essential oil were identified in dryland conditions. It was found that the highest amount of these compounds was related to isocamphopinone with a value of 38.63%. Moreover, compounds such as trans-pinocamphone (15.47%), ß-pinene (12.41%), ortho-menth-8-ene-4-methanol (6.02%), myrtenol (3.27%), thymol (2.92%) and pinocarvone (1.88%) had the highest amounts of essential oil composition compared to other essential oil compounds. Furthermore, it was found that among all the identified compounds in hyssop essential oil in irrigated conditions, the lowest amount of components was related to ά-Humulene, which had a value less than 1% among the constituents of hyssop essential oil (Table 3).

In the present study, the essential oil of hyssop flowers in both dryland and irrigated conditions was isolated and oil yields were obtained using the weight percentage of fresh flowers with three replications. Moreover, more than 95% of the compounds in hyssop essential oil in both irrigated and dryland conditions were identified. The highest amount of these compounds was related to isocamphopinone in both dryland and irrigated conditions. However, in the case of hyssop in irrigated conditions in 98.5% percent of the measured essential oil, about 38 compounds were identified, while with regard to hyssop flowers in dryland conditions in 97.39% of the essential oil, 41 compounds were identified. Among the significant identified compounds of hyssop in both irrigated and dryland conditions, isocamphopinone and thymol were more abundant than other compounds.

Essential oil compounds of Hyssop were different in both irrigated and dryland conditions, so that in this study isocamphopinone in hyssop essential oil in dryland conditions was higher than irrigated conditions, while in irrigated conditions, the trans-pinocamphone was higher than dryland conditions. Furthermore, at dryland conditions, increased isocamphopinone simultaneously occurred with reduced trans-pinocamphone (table 2 and 3).

However, in this study, it was found that irrigated conditions reduced other compounds of hyssop essential oil, for example, the amount of pinocarvone, which was 3.84% in irrigated conditions, reached 1.88% under dryland conditions and its amount was less than 50% in comparison with irrigated conditions. These results showed that the differences between irrigated and dryland conditions were very impressive and reflected the direct effect of drought on some compounds in herb hyssop.

The amount of thymol was 8.28% in irrigated conditions, whereas dryland conditions reduced the amount of this compound by 2.92% indicating a 65% decrease compared to irrigated conditions, which is very significant and shows the negative effects of drought on the reduction of this compound in hyssop plant. In this case, Dehghanzadeh *et al*., (15) reported that thirty–nine components were identified in the *H. officinalis* essential oil representing 99.82% of the total weight. They stated that the major components were thymol (18.95%), β-bisabolol (16.62%), carvacrol (7.73%), n-Dodecane (5.23%), caryophyllen (4.96%), ortho-acetanisol (4.72%), camphor (3.47%), cumin aldehyde (3.22%) and spathuleno (3.02%). Isopinocamphone (57.27%) was the major constituent of *H. officinalis* essential oil (16). Also, Wesolowska *et al*., (2) found that cis-pinocamphone (44.77%) is the main components of hyssop.

Other studies have shown the impact of drought on the essential oil compound of medicinal plants such as hyssop. For example, in a study conducted by Khosh Ighbal Gharabaei *et al*., (5) it was found that environmental conditions during the growing period affected on the essential oil compound of hyssop, which was consistent with the results of this study. They stated

Table 2: Essential Oil Compounds of Hyssop Flowers in Irrigated Conditions.

No	Component	%	RT	KI	Type
1	α-Thujene	0.11	10.97	927	MH
2	α-Pinene	0.26	11.35	935	MH
3	Sabinene	1.08	13.43	976	MH
4	β-Pinene	6.35	13.69	981	MH
5	1-Octen-3-ol	0.1	14	987	Other
6	β-Myercene	0.75	14.27	993	MH
7	O-Cymene	0.19	16.24	1031	MH
8	Limonene	0.57	16.4	1034	MH
9	β-Phellandrene	0.78	16.52	1036	MH
10	β-cis-Ocimene	0.09	17.28	1051	MH
11	Linalool	0.72	20.17	1107	MO
12	Cis-Thujene	0.1	20.6	1116	MO
13	Trans Thujene	0.17	21.2	1128	MO
14	Trans-Pinocarveol	0.19	22.39	1152	MO
15	Trans-Pinocamphone	19	23.49	1174	MO
16	Pinocarvone	3.84	23.58	1176	MO
17	Isocamphopinone	34.2	24.35	1192	MO
18	Myrtenol	4.17	25.17	1209	MO
19	Estragole	0.25	25.29	1211	Other
20	Thymyl methyl ether	0.09	27.02	1248	MO
21	Thymol	8.28	29.8	1307	MO
22	Carvacrol	0.36	30.16	1315	MO
23	1,5,5-Trimethyl-6-methylene-cyclohexene	0.17	31.09	1336	Other
24	β-Burbonene	0.58	33.48	1390	SH
25	β-elemence	0.11	33.72	1395	SH
26	α-Gurjunene	0.12	34.46	1412	SH
27	Methyl eugenul	0.67	34.57	1415	Other
28	Caryophyllene	0.69	35.07	1427	SH
29	α-Humulene	0.09	36.63	1464	SH
30	Allo-Aromadendrene	0.58	36.81	1469	SH
31	Germacrene D	1.5	37.7	1490	SH
32	Ortho-Menth-8-ene-4-methanol	6.86	40.53	1561	SO
33	Spathulenol	1.33	41.73	1591	SO
34	Caryophyllene oxide	0.16	41.92	1596	SO
35	Ledol	0.25	42.81	1620	SO
36	δ-Eudesmol	0.09	43.49	1638	SO
37	α-Cadinol	0.14	44.23	1657	SO
38	β-Eudesmol	0.85	44.79	1672	SO
	Total Identified	95.8			

that drought is one of the most important non-biological stresses that affect the growth, development and biochemical processes of plants such as the production of secondary metabolites and aromatic chemical compounds. Based on these results, a total of about 33 compounds of hyssop essential oil were identified, which represented 89% to 95% of the composition of hyssop essential oil. They also stated that the percentage of essential oil compounds increased in both levels of drought stress compared to the control and was higher in mild drought stress. However, it should be noted that the amount of essential oil in hyssop vegetative body varied between 0.3 until 1 percent. The hyssop vegetative body contains flavonoids and tannins at the rate of 5 to 8%, bitter substances at a rate of 3 to 4% and other substances such as diosmin, hisobin and mucilage compounds (17).

Table 3: Essential Oil Compounds of Hyssop Flowers in Dryland Conditions.

No	Component	%	RT	KI	Type
1	α-Thujene	0.33	10.97	927	MH
2	α-Pinene	0.75	11.35	935	MH
3	Camphene	0.14	12.24	952	MH
4	Sabinene	1.86	13.43	976	MH
5	β-Pinene	12.41	13.7	981	MH
6	1-Octen-3-ol	0.11	14	987	Other
7	β-Myercene	1.18	14.26	992	MH
8	α-Terpinene	0.2	15.74	1021	MH
9	ρ-Cymene	0.27	16.24	1031	MH
10	Limonene	0.81	16.4	1034	MH
11	β-Phellandrene	1.63	16.52	1036	MH
12	β-cis-Ocimene	0.17	17.28	1051	MH
13	δ-Terpinene	0.33	17.94	1064	MH
14	Trans-Sabinene-hydrate	0.3	18.67	1078	MO
15	α-Terpineneolene	0.1	19.31	1090	MH
16	Linalool	0.71	20.16	1107	MO
17	Cis-Thujene	0.13	20.6	1116	MO
18	Trans Thujene	0.15	21.19	1128	MO
19	Trans-Pinocarveol	0.16	22.39	152	MO
20	Trans-Pinocamphone	15.47	23.47	1174	MO
21	Pinocarvone	1.88	23.55	1176	MO
22	Isocamphopinone	38.63	24.34	192	MO
23	Myrtenol	3.27	25.16	1209	MO
24	Estragole	0.27	25.3	1211	Other
25	Thymol	2.92	29.78	1306	MO
26	Carvacrol	0.15	30.16	1315	MO
27	1,5,5-Trimethyl-6-methylene-cyclohexene	0.14	31.09	1336	Other
28	β-Burbonene	0.39	33.48	1390	SH
29	α-Gurjunene	0.09	34.46	1412	SH
30	Methyl eugenul	0.61	34.57	1415	Other
31	Caryophyllene	0.8	35.08	1427	SH
32	α-Humulene	0.09	36.64	1464	SH
33	Allo-Aromadendrene	0.44	36.81	1469	SH
34	Germacrene D	1.15	37.7	1490	SH
35	Bicyclogermacrene	1.11	38.31	1505	SH
36	Ortho-Menth-8-ene-4-methanol	6.02	40.53	1567	SO
37	Spathulenol	1.01	41.73	1591	SO
38	Caryophyllene oxide	0.13	41.93	1596	SO
39	Ledol	0.19	42.81	1620	SO
40	α-Cadinol	0.17	44.23	1657	SO
41	β-Eudesmol	0.72	44.97	1672	SO
	Total Identified	97.39			

Carvacrol which is one of the most important components of hyssop essential oil that can be greatly affected by low water available in dryland and also irrigated conditions was 0.15 and 0.36% in irrigated and dryland conditions respectively.
Their amounts in dryland were less than half of their rate in irrigated conditions. This reflects the fact that irrigated and dryland conditions can have different effects on the composition of the essential oil of hyssop and are able to change some of key components of the essential oils with high medical or industrial value. The status was similar for thymol, and it was observed that dryland conditions significantly reduced the amount of thymol in hyssop essential oil. However, the conditions for isocamphopinone were the opposite of thymol and carvacrol, and the amounts of isocamphopinone in irrigated and dryland conditions were 34.2 and 38.63% respectively, indicating that dryland conditions increased the amount of the predominant composition of hyssop essential oil, such isocamphopinone. The results also showed that the amounts of ß–pinene in irrigated and dryland conditions were 6.35 and 12.41% respectively, and it was found that the amount of ß–pinene in dryland conditions was about two times higher than irrigated conditions. The amount of limonene in hyssop essential oil was 0.57% in irrigated conditions, while its amount was about 0.81% in dryland conditions and was significantly increased in dryland compared to irrigated conditions. These results indicated an increase or decrease in some compounds in hyssop essential oil under varying water status. Moreover, it was indicated that dryland and irrigated conditions may not cause significant changes in some of the compounds in hyssop essential oil of and their amount was permanent in irrigated and dryland conditions. For example, in hyssop essential oil, the amounts of linalool in dryland and irrigated conditions were 0.71 and 0.72% respectively indicating that the stability of this component of hyssop essential oil and dry or irrigated conditions have no effect on it. Caryophyllene increased significantly in dry conditions in comparison with irrigated conditions. Hyssop essential oil is one of the most important herbal essential oils used in food and cosmetics products (2). Some researchers have also reported that essential oil compounds vary under irrigated and dryland conditions. In this case, Tavakoli and Aghajani (11) stated that ß–pinene increased in dryland conditions in comparison with irrigated conditions that was in line with the findings of the present study. Furthermore, they reported that linalool percentage increased and trans-pinocamphone decreased under dryland conditions. However, it was mentioned that medicinal plants react differently to dry conditions in terms of performance and production of effective materials. Hyssop is a highly significant medicinal plant because it has antibacterial, anti-cancer and antiviral properties (18). The main components of the essential oils were trans-pinocamphone, cis-pinocamphone and β- pinone. Hence, the essential oils obtained from the central regions of Iran are not similar to the ones of southern and western regions of the country, but rather similar to the components of the essential oil reported in Serbia (15). After the establishment of hyssop in Aligudarz region, we will be able to propose this plant for cultivation under degraded rangelands for medicinal goals.

Conclusion

Growing and essential oil components of medicinal plants such as hyssop in irrigation and dry land conditions were deferent. In the present study the essential oil compounds of the hyssop medicinal plant in irrigation condition was different in comparison with dry land condition, so that some compounds increased and others decreased, and some were constant. Moreover, depending on the production goal, hyssop can be cultivated in each of these conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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